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Improved liquid chromatography of salicylic acid and some related compounds on a phenyl column

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Abstract

Salicylic acid is used in many pharmaceutical and cosmetic formulations. In this study, salicylic acid (SA), three impurities: 4-hydroxybenzoic acid (4HBA), 4-hydroxyisophthalic acid (4HIPA), phenol (PHE), and two metabolites: gentisic acid (GA), salicyiglycine (SG) were chromatographed on both octadecylsilica (ODS) and phenyl high-performance liquid chromatography (HPLC) columns with UV detection. The effects of mobile phase pH and the addition of β-cyclodextrin to the mobile phase were investigated. Selectivity, resolution, and peak shape are discussed. Linearity, sensitivity, and precision studies were conducted. The phenyl column was compared to the ODS column for impurities testing of salicylic acid. Commercial products (a gel and a collodion) were assayed for salicylic acid using the proposed methodology.

Keywords: Phenyl column; Mobile phase composition; Salicylic acid; 4-Hydroxybenzoic acid; 4-Hydroxyisophthalic acid; Phenol; Gentisic acid; Salicylglycine; Cyclodextrins

1. Introduction

Salicylic acid is used as a topical keratolytic and as an external antiseptic and antifungal. Compendial testing of salicylic acid for impurities is accomplished with an ODS column and a mobile phase of methanol, water, and glacial acetic acid [1]. An ODS column and a mobile phase of methanol, acetonitrile, water, and glacial acetic acid have been used to analyze plasma and urine for salicylic acid and some of its metabolites [2]. In this study, salicylic acid and five related compounds are eluted from an ODS column under five different mobile phase conditions. This experiment is repeated using a phenyl column. The structures of the six test compounds are found in Fig. 1.

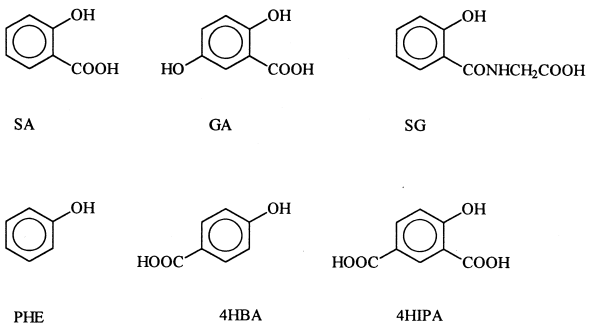

Fig. 1. Structures of test compounds.

2. Experimental

2.1. Reagents

Methanol was HPLC grade. All other chemicals

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were reagent grade. Potassium hydroxide, salicylglycine, and 4-hydroxybenzoic acid were obtained from Acros Organics (Pittsburgh, PA, USA). Phenol and 4-hydroxyisophthalic acid were purchased from Aldrich (Milwaukee, WI, USA). Glacial acetic acid and phosphoric acid were obtained from J.T. Baker (Phillipsburg, NJ, USA). Salicylic acid and methanol were purchased from Fisher (Fairlawn, NJ, USA). Gentisic acid was obtained from Lancaster Synthesis (Windham, NH, USA). β-cyclodextrin was purchased from TCI (Portland, OR, USA). Water was obtained from a Millipore (Milford, MA, USA) MilliQ reagent water system.

2.2. Instrumentation

Chromatography was performed on a Hewlett–Packard (Wilmington, DE, USA) model 1100 isocratic pump. Samples were injected by means of a Waters (Milford, MA, USA) 717 autosampler and detection was accomplished by means of a Waters 486 variable wavelength detector. Chromatograms were processed by Waters ExpertEase software (Version 3.2).

2.3. Chromatographic conditions

Samples were injected onto a 5-micron, 250×4.6-mm, Zorbax (Chadds Ford, PA, USA) ODS column or a Zorbax StableBond phenyl column. Analytes were eluted with one of the following mobile phases at a flow-rate of 1.0 ml/mm:

Mobile Phase (A) methanol–water–glacial acetic acid, 40:60:1, v/v/v, pH=3.0.

Mobile Phase (B) methanol–water–glacial acetic acid, 40:60:1, v/v/v, containing 2.5 g β-cyclodextrin per liter, pH=3.0.

Mobile Phase (C) methanol–water–phosphoric acid, 40:60:1, v/v/v, the pH is adjusted to 2.0 with potassium hydroxide pellets.

Mobile Phase (D) methanol–water–phosphoric acid, 40:60:1, v/v/v containing 2.5 g β-cyclodextrin per liter, the pH is adjusted to 2.0 with potassium hydroxide pellets.

Mobile Phase (E) methanol–water–phosphoric acid, 40:60:1, v/v/v containing 5.0 g β-cyclodextrin per liter, the pH is adjusted to 2.0 with potassium hydroxide pellets.

UV detection occurred at 285 nm, except where noted.

2.4. Resolution study

Twenty microliters of a methanolic solution of the six test compounds (each at 10 μg/ml) were injected. Chromatograms of the test mixture, injected on ODS and phenyl columns using the five mobile phases are found in Figs. 2–6.

2.5. Linearity study

A stock solution containing the six analytes was diluted to yield seven concentrations of the test compounds (0.1, 0.5, 1, 5, 10, 50, and 100 μg/ml). Twenty microliters of each methanolic dilution were injected onto a phenyl column and eluted with mobile phase D.

2.6. Impurities testing

A standard solution was prepared as directed in USP 23 for impurities testing of salicylic acid [1]. Two microliters of the standard were injected on an

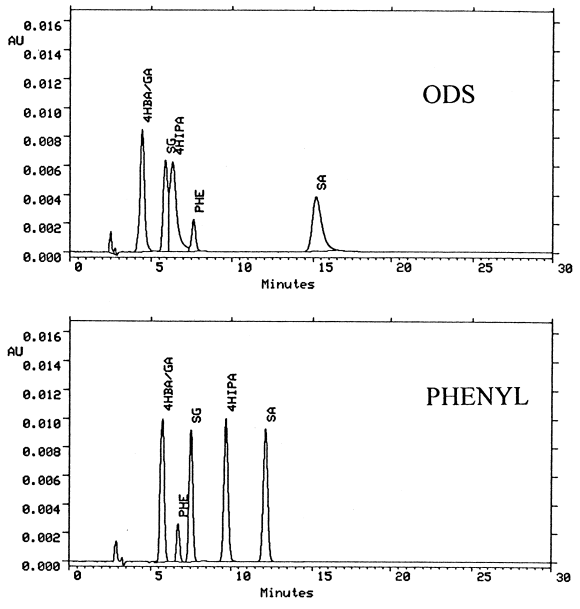

Fig. 2. Test mixture eluted with mobile phase (A) methanol–water–glacial acetic acid, 40:60:1, v/v/v, pH=3.0.

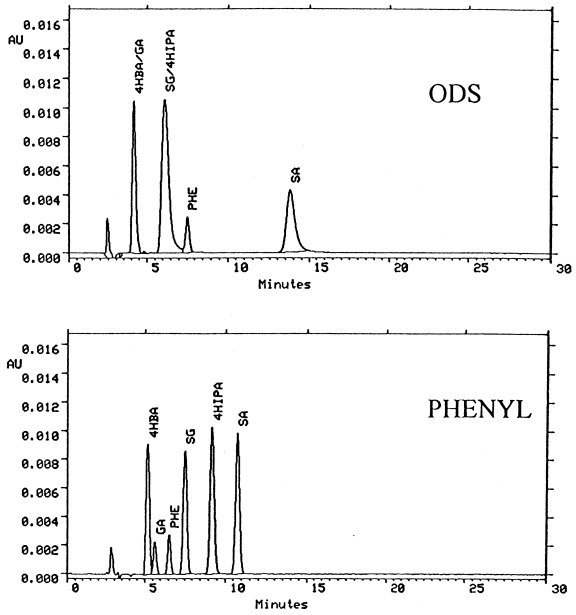

Fig. 3. Test mixture eluted with mobile phase (B) methanol–water–glacial acetic acid, 40:60:1, v/v/v, containing 2.5 g β-cyclodextrin per liter, pH=3.0.

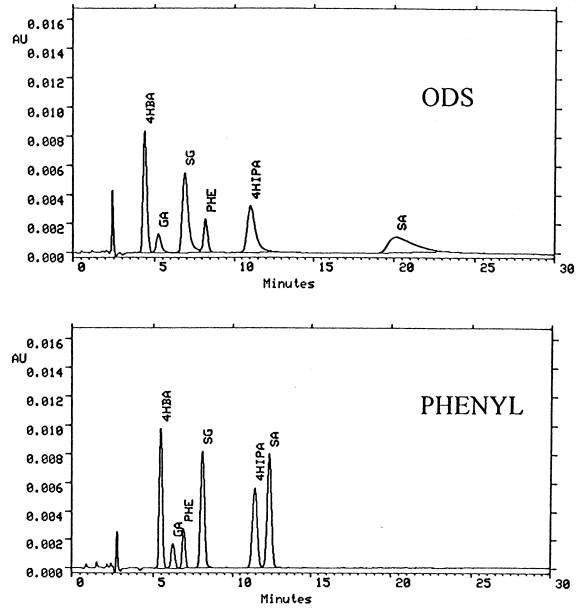

Fig. 5. Test mixture eluted with mobile phase (D) methanol–water–phosphoric acid, 40:60:1, v/v/v, containing 2.5 g β-cyclodextrin per liter, the pH is adjusted to 2.0 with potassium hydroxide pellets.

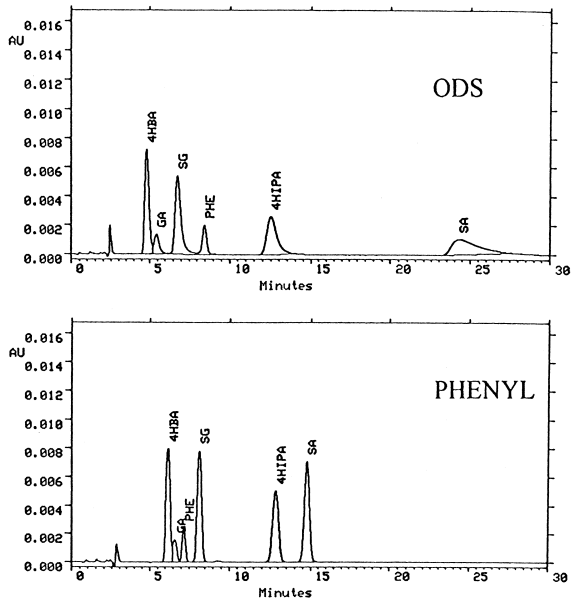

Fig. 4. Test mixture eluted with mobile phase (C) methanol–water–phosphoric acid, 40:60:1, v/v/v, the pH is adjusted to 2.0 with potassium hydroxide pellets.

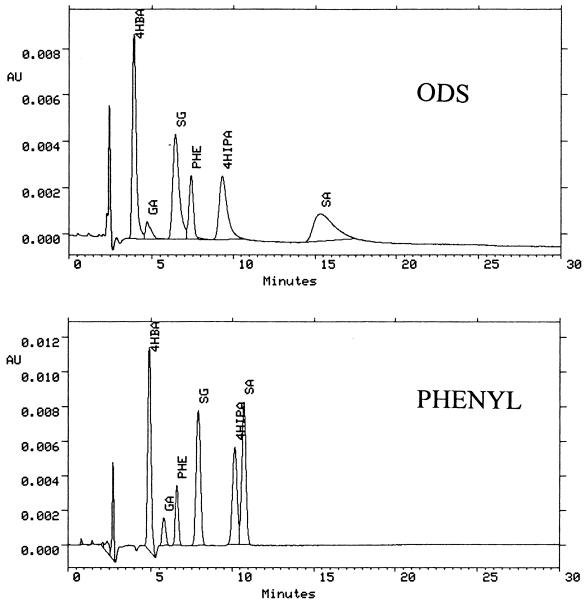

Fig. 6. Test mixture eluted with mobile phase (E) methanol–water–phosphoric acid, 40:60:1, v/v/v, containing 5.0 g β-cyclodextrin per liter, the pH is adjusted to 2.0 with potassium hydroxide pellets.

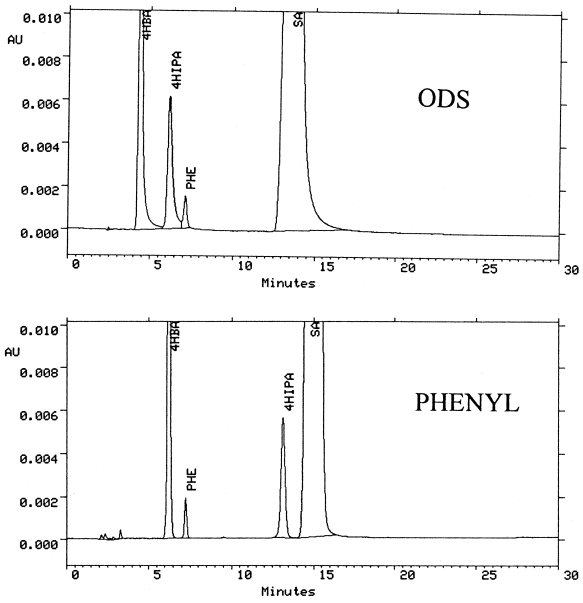

Fig. 7. Standard for impurities testing of salicylic acid.

ODS column and eluted with mobile phase A. The experiment was repeated on a phenyl column using mobile phase C for elution. Analytes were detected at 270 nm. Chromatograms obtained on the two columns are shown in Fig. 7.

2.7. Assay of commercial products

A salicylic acid gel and a salicylic acid collodion product were diluted in methanol 1 to 1000 and 1 to 10 000 respectively. Diluted samples were injected onto a phenyl column and salicylic acid was eluted with mobile phase D. Concentrations of salicylic acid were determined from a calibration curve.

3. Results and discussion

3.1. Resolution study

In Fig. 2, 4HBA and GA were co-eluted at pH 3 (mobile phase A) on both the ODS and phenyl columns. An elution order of 4HBA/GA, SG, 4HIPA, PHE, and SA, was obtained on ODS; while on phenyl, the elution order was 4HBA/GA, PHE, SG, 4HIPA, and SA.

Addition of β-cyclodextrin at pH 3 (mobile phase B) did not resolve 4HBA and GA on the ODS column as seen in Fig. 3. The analytes SG and 4HIPA were co-eluted on the ODS column. The retention times of some analytes were reduced on both columns.

By changing the mobile phase pH to 2 (mobile phase C), 4HBA and GA showed partial resolution and baseline resolution was seen for all other peaks on both columns (Fig. 4). Retention times have increased for all analytes. The elution order on ODS was 4HBA, GA, SG, PHE, 4HIPA, and SA. For phenyl the elution order was 4HBA, GA, PHE, SG, 4HIPA, and SA. Peak tailing for SA was significantly more pronounced on the ODS column than the phenyl column.

By using a pH 2 mobile phase containing 0.25% β-cyclodextrin (mobile phase D), baseline resolution was obtained for all six analytes on both columns (Fig. 5). Tailing continued for SA on the ODS column.

Increasing the β-cyclodextrin content to 0.50% at pH 2 (mobile phase E), resulted in some loss of resolution for SG and PHE on the ODS column and 4HIPA and SA on the phenyl column (Fig. 6). The peak shape of SA on the ODS column showed improvement.

Overall improvement in resolution, peak shape, and peak tailing was observed for the phenyl column over ODS. While shorter run times were obtained on the phenyl column, selectivity advantages were offered on both columns. The pK_a of GA and SA is 2.97. In a pH 2 mobile phase, ionization of these two analytes will be greatly reduced. The mobile phase pH and the pK_a of the analytes played important roles in both selectivity and resolution [3]. The mobile phase component β-cyclodextrin was instrumental in separating six analytes that are very similar in structure.

3.2. Linearity, sensitivity, precision

The six analytes were found to be linear over a range of 1 to 100 μg/ml (Table 1). The limit of detection ($S/N=3$) for the test compounds: 4HBA, GA, PHE, SG, 4HIPA, and SA was 0.15, 0.15, 0.12, 0.08, 0.10, and 0.07 μg/ml, respectively. The peak areas of the test compounds showed an intra-day and

Table 1
Linearity of test compounds

Component	Slope	*y*-Intercept	Correlation coefficient
4HBA	15142	−4339	0.9998
GA	2460	174	0.9997
PHE	4293	−322	0.9997
SG	14849	−5206	0.9995
4HIPA	11504	−3968	0.9995
SA	14979	−5327	0.9995

Five concentrations of each analyte were used for the regression. Slope in terms of peak area counts μg^{-1} ml^{-1}. Intercept in terms of peak area counts.

an inter-day (mean peak areas) relative standard deviation of less than 2% (Table 2).

3.3. Impurities testing

The standard concentrations for the three impurities: 4HBA, 4HIPA, and PHE are 50, 25, and 10 μg/ml, respectively. These levels are within the linear range of the proposed methodology. The phenyl column with mobile phase C demonstrated resolution and sensitivity comparable to the compendial method for impurities in salicylic acid.

Table 2
Precision of test compounds

Component	Intra-day (n=21) R.S.D. (%)	Inter-day (n=4) R.S.D. (%)
4HBA	0.46	0.93
GA	0.54	0.31
PHE	0.77	1.20
SG	0.51	0.27
4HIPA	0.47	0.27
SA	0.54	0.42

Inter-day was the mean area of 21 daily injections.

Table 3
Assay of commercial products

Product type	Label claim (%SA)	Found (%SA)	Spike sample (%Recovery)
Gel	2.00	1.95	100.5
Collodion	17.00	17.77	103.1

3.4. Analysis of commercial products

Assay results for a salicylic acid gel and a salicylic acid collodion product along with recovery data are given in Table 3. Titration is the compendial method for salicylic acid in collodion and gel [4,5]. The simple and rapid HPLC procedure presented in this paper might be developed into a stability indicating method for salicylic acid in collodion and gel products.

Acknowledgements

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